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Evaluation of In-Vitro Anti-Oxidant and Anti-Ulcer Activity of Karuvepillai Vadagam Extract Against Aspirin Induced Ulcer Model in Albino Wistar Rats

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ABSTRACT

The aim of this study is to evaluate the anti-oxidant and anti-ulcer activity of karuvepillai vadagam extract (KVP) in aspirin induced ulcer model in albino wistar rats. Either sex of albino wistar rats was separated into five groups, each consisting of n=6. *In-vitro* antioxidant activity of Karuvepillai vadagam extract was evaluated by DPPH radical scavenging assay and hydrogen peroxide radical scavenging assay exhibited good anti-oxidant activity. *In-vivo* anti-ulcer activity was evaluated by aspirin-induced ulcer model. Group I received normal feed and water, group II received aspirin 400 mg/kg on 21st day of study, Group III received omeprazole 20 mg/kg and Group IV and V received test drug (200 mg/kg of KVP and 400 mg/kg of KVP, bd.wt, p.o) for 21 days. On 21st day, aspirin (400 mg/kg, bd.wt, p.o) was administered to all groups other than normal group with prior fasting of 24 hours. Following treatment, the anti-ulcer effect of KVP was evaluated by biochemical, macroscopic & histopathological examination among the experimental groups. The gastric volume, pH, total acidity and free acidity were evaluated in biochemical parameters. The ulcer index and percentage inhibition of ulcer were evaluated by macroscopic examination. The gastric volume, total acidity, free acidity and ulcer index were decreased significantly ($P<0.01$ & $P<0.0001$) in standard and treatment groups while pH of the gastric content was increased significantly ($P<0.0001$) compared with the disease control group. In histopathological examination, there was normal gastric epithelium and reduction in inflammation in drug-treated groups.

1. INTRODUCTION

An ulcer primarily affects the stomach and the upper part of the small intestine. It is caused by a lack of balance between the gastric aggressive factors and gastric protective factors. Aggressive factors include increased secretion of HCL and pepsin, inadequate dietary habits, free oxygen radicals, long-term use of NSAIDs, smoking, excessive alcohol consumption, high-stress levels and infection of helicobacter pylori. Gastric protective factors include adequate gastric blood flow, secretion of prostaglandin, mucous nitric oxide, bicarbonate, and growth factors.[9] Genetic factors also play a role in the pathogenesis of ulcer disease. The common symptoms include abdominal pain, indigestion, nausea and vomiting, loss of appetite and weight loss.[1][2]

In aspirin-induced ulcer model, aspirin works by inhibiting the activity of an enzyme called cyclooxygenase (COX), specifically COX-1 which is responsible for the production of prostaglandins, which play a protective role in the gastrointestinal (GI) tract by promoting mucus secretion, enhancing blood flow, and maintaining the integrity of the stomach lining. By inhibiting COX-1, aspirin reduces the production of prostaglandins, leading to a decrease in the protective factors that help prevent ulcer formation. Aspirin can stimulate the secretion of gastric acid, which further contributes to the erosion of the stomach lining. Increased acid production, combined with the weakened mucosal barrier, creates an environment that is more prone to ulcer formation.[1][2]

The treatment of ulcers aims to relieve symptoms, promote healing, and prevent complications such as antibiotics for H. pylori infection, acid-suppressing medications like proton pump inhibitors (PPIs) like omeprazole and histamine receptor blockers (H2 blockers) like ranitidine are commonly used to reduce stomach acid production, cytoprotective agents (sucralfate), coat the ulcer and protect it from further damage by forming a protective barrier.[3][4]

Lifestyle modifications: Individuals with ulcers are advised to make certain lifestyle changes, such as avoiding irritants like spicy foods, alcohol, and tobacco. Managing stress levels through relaxation techniques can also be beneficial.

Prolonged use of these drugs may lead to serious adverse effects like thrombocytopenia, nephrotoxicity, hepatotoxicity and impotence. Due to unwanted side effects of existing anti-ulcer drugs, there is need to be further effective and safe treatment for ulcers.

Karuvepillai Vadagam is a polyherbal formulation containing leaves of ***Murraya koenigii***, aril of ***Myristica fragrance***, seeds of ***Coriander sativum***, seeds of ***Pipper nigrum***, root of ***Curcuma zedoaria*** and salt has been chosen as the drug to perform the research after ethanolic extraction on its anti-ulcer action based on the literature review. The use of polyherbal formulation is used to reduce the side effects of anti-ulcer drugs such as abdominal pain or discomfort, constipation, indigestion, diarrhea, flatulence, nausea, vomiting and impotence.[3]

2. MATERIALS AND METHODS

2.1 Procurementod drug and extraction process

The drug Karuvepillai vadagam was procured from Captain Srinivasa Murthy Regional Ayurveda Research Drug Development Institute, Arumbakam, Chennai. The standard drug Omeprazole 20 mg and the inducing drug Aspirin were procured from a retail pharmacy manufactured by Cipla Ltd and then KVP sample was extracted with ethanolic solvent by using a soxhlet apparatus.

2.2 *IN-VITRO* ANTI-OXIDANT ACTIVITY [13-19]

2.2.1 Principle DPPH radical scavenging assay

The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an anti-oxidant. It measures compounds that are radical scavengers, below reaction shows the mechanism by which DPPH accepts hydrogen from an antioxidant. DPPH is one of the few stable and commercially available organic nitrogen radicals. The antioxidant effect is proportional to the disappearance of DPPH in test samples. Monitoring DPPH with a UV spectrometer has become the most commonly used method because of its simplicity and accuracy. DPPH shows a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517 nm.

Procedure

1. Prepare 0.1 mM of DPPH solution in methanol and add 100 µl of this solution to 300 µl of the solution of the test drug at different concentrations (500, 250, 100, 50, and 10 µg/ml).

2. The mixtures have to be shaken vigorously and allowed to stand at room temperature for 30 minutes.

3. Then the absorbance has to be measured at 517 nm using a UV-visible spectrophotometer. (Ascorbic acid can be used as the reference).

4. Lower absorbance values of the reaction mixture indicate higher free radical scavenging activity.

5. The capability of scavenging the DPPH radical can be calculated by using the following formula.

$$\text{DPPH scavenging effect (\% inhibition)} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Absorbance of control}} \times 100$$

Where,

Abs control – Absorbance of the DPPH radical

Abs sample – Absorbance of DPPH + Karuvepillai vadagam

The antioxidant activity of KVP will be expressed as IC50 and compared with the standard.

2.2.2 Principle of Hydrogen peroxide radical scavenging assay

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H2O2 can probably react with Fe2+, and possibly Cu2+ ions to form hydroxyl radicals and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate.

Procedure

1. A solution of hydrogen peroxide (43 mM) is prepared in phosphate buffer (1 M pH 7.4).

2. Different concentration of sample KVP (500, 250, 100, 50 and 10 µg/ml) was added to a hydrogen peroxide solution (0.6 ml, 43 mM).

3. Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide.

4. Ascorbic acid was used as standard. The free radical scavenging activity was determined by evaluating the % inhibition.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{absorbance of sample X 100}}{\text{Absorbance of control}}$$

Where,

Abs control – Absorbance of the DPPH radical

Abs sample – Absorbance of DPPH + Karuvepillai vadagam

The antioxidant activity of KVP extract was expressed as IC_{50} and compared with the standard.

2.3 Animal Procedure:

The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee and this protocol met the requirements of national guidelines of CPCSEA/IAEC approval no: 1917/GO/ReBi/S/16/CPCSEA/25.10.2016 and 05/AEL/IAEC/MMC, Date: 14.12.2022. For this investigation, 27 Wistar albino rats were purchased from the Madras Medical College Animal House in Chennai, India. In a quarantine period, animals are kept apart from those already housed in the facility while their health as well as their microbiological condition are being assessed. The newly procured Wistar albino rats were quarantined for a period of one week to minimize the chance of introduction of pathogens into established animals and allowed to develop psychological, physiological and nutritional stabilization before their use. The animals were housed in a well-ventilated animal house which was maintained at a constant temperature and relative

humidity of 55 to 60%. The animals were housed in spacious polypropylene cages and paddy husk was utilized as bedding material. The bed material was changed twice a week. The animals were maintained on standard pellets and purified water. The animals were provided with food ad libitum except during fasting. All animal cages used in the study had proper identification i.e., labels. Each animal in the cage was marked on the tail with picric acid for their appropriate identification.

2.4 *ACUTE TOXICITY*

Since it is an herbal formulation a limit test has been performed with 3 animals at a dose of 2000mg/kg according to OECD guideline 423 (Acute oral toxicity) for about 14 days for dose selection. [10]

2.5 *ANTI-ULCER ACTIVITY*

Animals were fasted 8 hours before the start of the study but provided with drinking water *ad libitum*. Group I received normal feed and water, Group II received aspirin 400 mg/kg on 21st day only, Group III received omeprazole 20 mg/kg and Group IV and V received test drugs (200 and 400 mg/kg, bd.wt, *p.o*) for 21 days. On 21st day, aspirin (400mg/kg, bd.wt, *p.o*) was administered to all animals other than the normal group with prior fasting of 24 hours. [5-11]

2.6 *EVALUATION OF ANTI-ULCER ACTIVITY*

2.6.1 Biochemical Parameters

After completion of the experimental period (21 days), the rats were fasted for 24 hours then the rats were sacrificed on 22nd day by using 2% isoflurane. All the animals of the stomach were opened along with the greater curvature and gastric content were collected for estimation of biochemical parameters like estimation of gastric volume, evaluation of pH, estimation of total acidity and free acidity. Macroscopic evaluations like ulcer index and percentage inhibition of ulcer and stomach tissue were immersed in 10% formalin solution for histopathological studies. [7-9]

2.7 Histopathological Examination

Stomach was isolated from the rats in all six groups and then fixed in 10 % neutral buffered formalin for 24 hours. The tissues were cleaned with methyl benzoate after drying using a

graded series of alcohols and embedded in wax with paraffin. Stomach sections were cut, and stained with hematoxylin and eosin dissolved in 95 % ethanol was used to stain the counter. Stomach sections were observed under a microscope after dehydration and clearing. [11,12,20]

2.8 Statistical Analysis:

All the values were expressed as mean ± SEM. Graph Pad Prism Software version 9.5.2 was used to statistically analyze the data using one-way ANOVA and Dunnett's multiple comparison test. P values were regarded as statistically significant if they were between 0.05 and 0.001.

3. RESULTS AND DISCUSSION

3.1 Invitro antioxidant activity

In-vitro antioxidant activity was evaluated by DPPH radical scavenging assay and hydrogen peroxide radical scavenging assay. Based on the results of the DPPH free radical scavenging assay presented in **Table no 1 and Figure 1,** the percentage inhibition of ascorbic acid was found to be **88.7%** and the sample (KVP) gives a maximum of **84.51%** inhibition. The IC_{50} value of standard ascorbic acid was found to be **49.82 µg/ml**, and IC_{50} value of KVP sample was found to be **67.2 µg/ml**. The results of the hydrogen peroxide radical scavenging assay presented in the **Table no 2 and Figure 2,** the percentage inhibition of ascorbic acid was found to be **82.9%** and the sample (KVP) gives a maximum of **81.5%** inhibition. The IC_{50} value of standard ascorbic acid is found to be **51.4 µg/ml**, and IC_{50} value of KVP sample was found to be **79.8 µg/ml**. In both the assay karuvepillai vadagam extract and standard ascorbic acid exhibited good antioxidant activity.

Table 1. Percentage inhibition and IC_{50} value of the sample by DPPH radical scavenging assay

S. No	Sample concentration (µg/ml)	OD Value at 517 nm (in triplicates)			% Inhibition of the sample	IC_{50} value
1.	Ascorbic acid	0.118	0.111	0.105	88.7086	49.82 µg/ml
2.	500 µg/ml	0.14	0.177	0.141	84.5166	67.2 µg/ml
3.	250 µg/ml	0.362	0.393	0.367	62.069	
4.	100 µg/ml	0.528	0.521	0.543	46.1799	
5.	50 µg/ml	0.771	0.797	0.767	21.0615	
6.	10 µg/ml	0.83	0.841	0.834	15.3144	
7.	Control	0.985	0.987	0.988	-	-

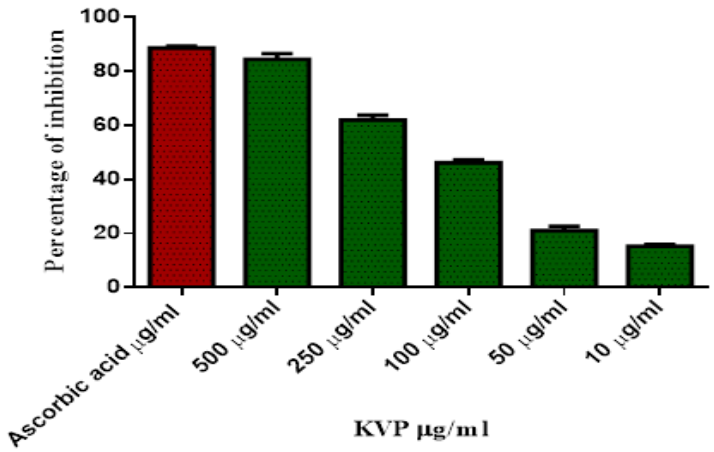

Figure: 1 – Graphical representation of percentage inhibition of ascorbic acid vs. KVP

Table 2. Percentage inhibition and IC_{50} value of the sample by Hydrogen peroxide radical scavenging assay

S. No	Sample concentration (µg/ml)	OD Value at 230 nm (in triplicates)			% Inhibition of the sample	IC_{50} value
1.	Ascorbic acid	0.131	0.134	0.132	82.90589	51.4 µg/ml
2.	500 µg/ml	0.121	0.131	0.135	81.51174	79.8 µg/ml
3.	250 µg/ml	0.209	0.208	0.204	72.10896	
4.	100 µg/ml	0.222	0.267	0.262	65.04567	
5.	50 µg/ml	0.421	0.433	0.435	41.20447	
6.	10 µg/ml	0.517	0.519	0.523	30.04821	
7.	Control	0.736	0.751	0.727	-	-

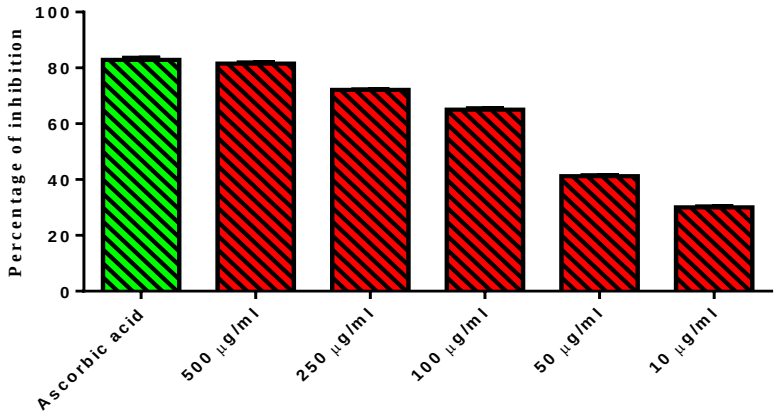

Figure 2. Graphical representation of percentage inhibition of ascorbic acid vs. KVP

3.2 *In-Vivo* Acute Toxicity Studies:

The Acute Oral toxicity is performed as per OECD guideline 423. There was no mortality and morbidity detected up to a dose of 2000 mg/kg in Wistar albino rats during a 14-day period of KVP extract administration. Acute toxicity studies help to determine the safety of substances when administered in a single high dose, while the 14-day period allows for evaluation of potential sub-acute effects. Hence 1/5th and 1/10th, a dose of **200 mg/kg** and **400 mg/kg** were designated as the low dose and high dose of KVP extract, respectively.

3.3 *In-vivo* anti-ulcer activity by aspirin-induced ulcer model:

3.3.1 *Effect of Karuvepillai vadagam on biochemical parameters:*

Aspirin-treated rats results increased gastric volume on the 22nd day compared to that of normal control rats which demonstrated that aspirin affects the prostaglandin synthesis increases the gastric volume. On treatment with omeprazole (20mg/kg) and Karuvepillai vadagam at the dose of 200mg/kg and 400 mg/kg showed significant changes in gastric volume. Treatment with the standard group showed decreased gastric volume significantly ($P < 0.0001$). It also showed a significant ($P< 0.01$ and $P<0.0001$) decrease in the level of gastric volume in the low and high-dose treated group when compared with disease control rats as shown in Table 2.

The pH of the gastric content decreased in aspirin-treated rats when compared with the normal control group of rats. When treated with omeprazole there was a significant ($P< 0.0001$) increase in pH levels. Karuvepillai vadagam showed a significant ($P < 0.0001$) increase in the pH level compared with disease control rats as shown in Table 2. These results confirmed that aspirin decreased the pH and increased the gastritis in aspirin-induced ulcers. Karuvepillai vadagam reduces the gastritis.

The total acidity of the gastric content increased in aspirin-treated rats when compared with the normal control group of rats. When treated with omeprazole there was a significant ($P < 0.01$) decrease in total acidity levels. Karuvepillai vadagam showed a significant ($P < 0.01$) decrease in the total acidity compared with disease control rats as shown in Table 2.

The free acidity of the gastric content increased in aspirin-treated rats when compared with the normal control group of rats. When treated with omeprazole there was a significant ($P < 0.0001$) decrease in free acidity levels. Karuvepillai vadagam showed a significant ($P < 0.01$ and $P < 0.0001$) decrease in the free acidity compared with disease control rats as shown in Table 2. These results confirmed that aspirin increased the acidity in aspirin-induced ulcers. Hence Karuvepillai vadagam reduces the acidity in the stomach.

Table 3. Biochemical parameters of gastric contents.

Groups	Gastric volume	pH	Total acidity	Free acidity
Normal	1.16±0.04	2.8±0.04	12.6±0.05	7.1±0.05
Disease Control	3.4±0.07[a]	1.7±0.08[a]	50.5±0.1[a]	36.3±0.05[a]
Standard control (Omeprazole 20 mg/kg)	2.13±0.04[c]	3.5±0.04[c]	19.03±0.05[b]	12.7±0.1[c]
Low dose (200 mg/Kg of KVP)	3±0.12[b]	3.06±0.04[c]	36.7±0.05[b]	30±0.05[b]
High dose (400 mg/Kg of KVP)	2.53±0.04[c]	3.3±0.04[c]	23.8±0.05[b]	22.8±0.15[c]

[a] P < 0.0001 compared with normal control.

[b] P < 0.01 compared with disease control.

[c] P< 0.0001 compared with disease control.

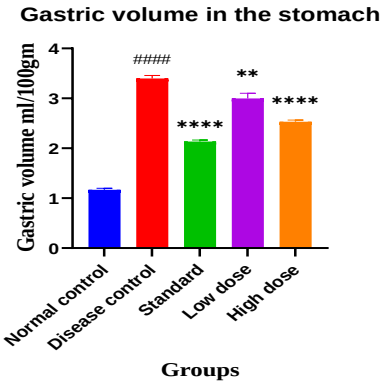

Figure 3a. Gastric volume

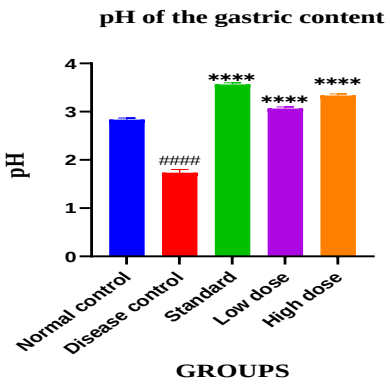

Figure 3b. pH of gastric content

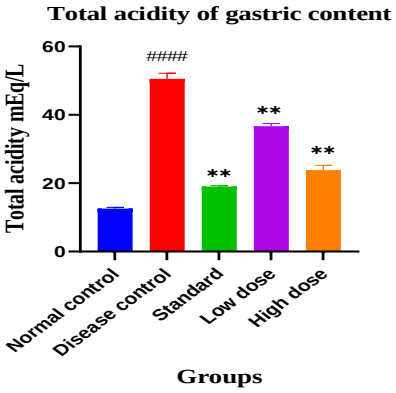

Figure 3c. Total acidity of gastric content

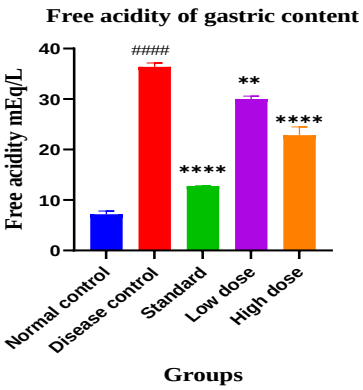

Figure 3d. Free acidity of gastric content

Figure 3. Effect of KVP on biomechanical parameters.

All the Values are plotted as mean ± SEM (n=3). Analyzed by One-way analysis of variance (ANOVA) followed by multiple comparisons Dunnet's t-test. #### P < 0.0001 compared with normal control; ****P < 0.0001 and **P < 0.01 compared with disease control.

3.3.2 *Effect of Karuvepillai vadagam on macroscopic evaluation:*

After macroscopic evaluation ulcer index was calculated, as there was an increased ulcer index in aspirin-treated rats when compared with the normal control group of rats. When treated with omeprazole there was a significant (P < 0.0001) decrease in ulcer index. Karuvepillai vadagam showed a significant (P< 0.0001) decrease in the ulcer index compared with disease control rats as shown in Table 2.

From the values of the ulcer index, the percentage inhibition of the ulcer was calculated. The ulcer protection in the standard omeprazole (20 mg/kg) group was **59.4%.** In Karuvepillai vadagam treated groups of 200 mg/kg and 400 mg/kg were **34.7%** and **50.4%** respectively. Among both low-dose and high-dose groups, high dose increased the percentage of ulcer protection more effectively than low dose group but not as much as the standard group.

Table 4. Macroscopic evaluation of ulcer index and % inhibition of ulcer

Groups	**Ulcer index**	**% protection of ulcer**
Normal	8.3±0.05	-
Disease Control	27.9±0.1[a]	-
Standard control (Omeprazole 20 mg/kg)	11.3±0.05[b]	59.4%
Low dose (200 mg/Kg of KVP)	18.2±0.05[b]	34.7%
High dose (400 mg/Kg of KVP)	13.8±0.05[b]	50.4%

[a] P < 0.0001 compared with normal control.

[b] P < 0.0001 compared with disease control.

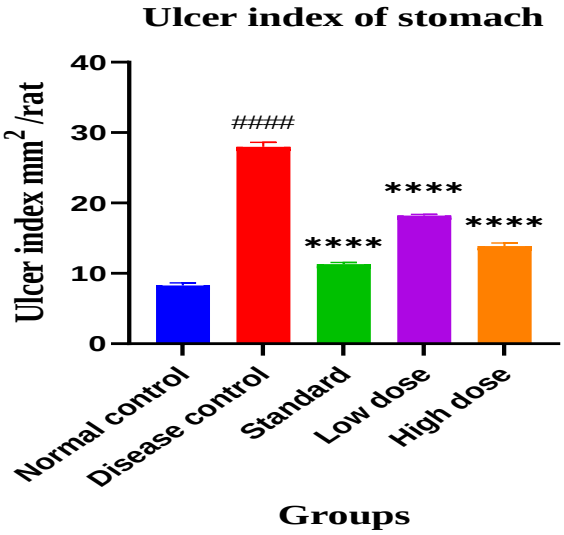

Figure 4. Effect of KVP on ulcer index.

All the Values are plotted as mean ± SEM (n=3). Analyzed by One-way analysis of variance (ANOVA) followed by multiple comparisons Dunnet's t-test. #### P < 0.0001 compared with normal control; ****P < 0.0001 compared with disease control.

3.4 Histopathological Examination:

The histopathological examination was performed in different groups using hematoxylin and eosin stain and is shown in Figures 3, 4 & 5 respectively.

Histopathological analysis of rat stomach tissue revealed normal gastric epithelium in normal control, while increased epithelial damage, hyperplasia of the gastric epithelium, destruction of gastric pits by inflammatory cells, and hypertrophy of the muscular layer in the disease control group. In contrast, the standard control group displayed normal gastric epithelium with focal gland destruction and a normal muscular layer. The treatment with both low-dose group 200 mg/kg of KVP and high-dose group 400 mg/kg of KVP shows normal gastric epithelium with focal gastric gland destruction by inflammation and normal muscular layer compared to the disease control, while the high dose demonstrating a more significant reduction in inflammation and shown normal gastric epithelium than low dose of KVP.

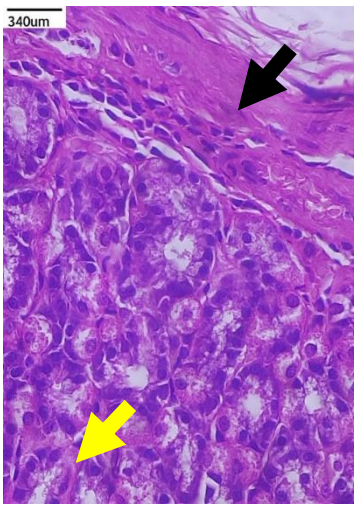

Figure 5a. Normal control

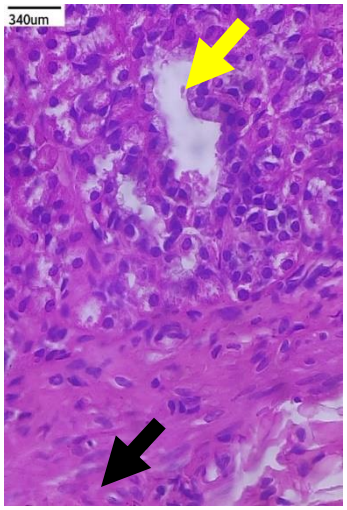

Figure 5b. Disease control (Aspirin)

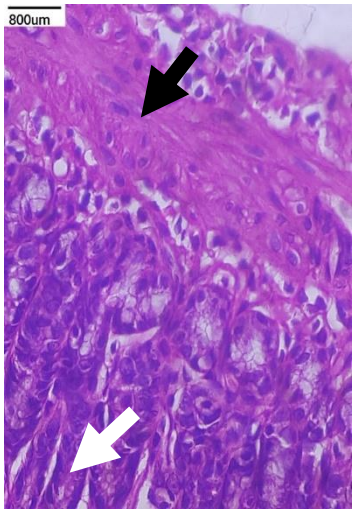

Figure 5c. Standard control (Omeprazole)

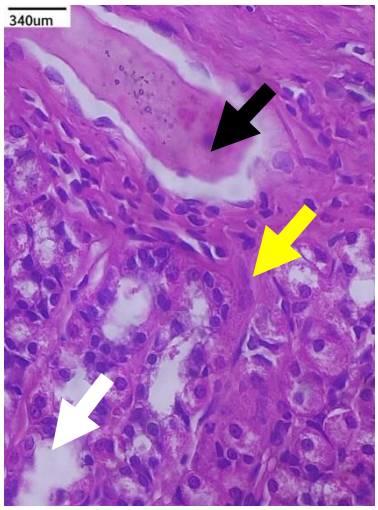

Figure 5d. Treatment group (200 mg/kg of KVP)

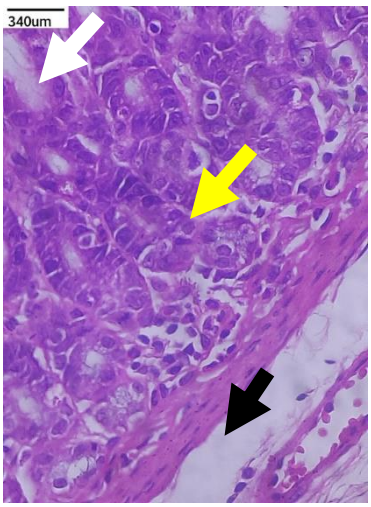

Figure 5e. Treatment group (400 mg/kg of KVP)

Figure 5. Histopathological examination using H&E stain. Gastric epithelium (white arrow), muscular layers (black arrow), inflammatory cells (yellow arrow).

CONCLUSION

From the study, it is concluded that the KVP possesses a beneficial effect against aspirin-induced ulcer proved by the valid data obtained from the in-vitro and in-vivo evaluation which includes antioxidant potential, biochemical parameters, macroscopic evaluation and

histopathological examination. Further research and clinical trials are crucial to validate the mechanism of action, efficacy, and safety of a particular intervention or treatment in human subjects. Validating the mechanism of action involves understanding how the treatment works at a biological or physiological level. These trials will provide crucial evidence to support the adoption of new treatments, ensure patient safety and improve overall healthcare outcomes.

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REFERENCES

[1] Huang, J. Q., Sridhar, S., & Hunt, R. H. (2002, January). Role of Helicobacter pylori infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. The Lancet, 359(9300), 14–22. https://doi.org/10.1016/s0140-6736(02)07273-2

[2] Take, S., Mizuno, M., Ishiki, K., Nagahara, Y., Yoshida, T., Yokota, K., Oguma, K., Okada, H., & Shiratori, Y. (2005, May). The Effect of Eradicating Helicobacter Pylori on the Development of Gastric Cancer in Patients with Peptic Ulcer Disease. The American Journal of Gastroenterology, 100(5), 1037–1042. https://doi.org/10.1111/j.1572-0241.2005.41384.x

[3] Chan, F. K., & Leung, W. (2002, September). Peptic-ulcer disease. The Lancet, 360(9337), 933–941. https://doi.org/10.1016/s0140-6736(02)11030-0

[4] Jones, M. P. (2006, April). The role of psychosocial factors in peptic ulcer disease: Beyond Helicobacter pylori and NSAIDs. Journal of Psychosomatic Research, 60(4), 407–412. https://doi.org/10.1016/j.jpsychores.2005.08.009

[5] Kauffman, G. (1989, February). Aspirin-Induced Gastric Mucosal Injury: Lessons Learned From Animal Models. Gastroenterology, 96(2), 606–614. https://doi.org/10.1016/s0016-5085(89)80056-3

[6] Gul, H., Abbas, K., & Qadir, M. I. (2015, March 19). Gastro-protective effect of ethanolic extract of Mentha longifolia in alcohol and aspirin-induced gastric ulcer models. Bangladesh Journal of Pharmacology, 10(1). https://doi.org/10.3329/bjp.v10i1.21689

[7] Roy, C., & Das, S. (2012). The protective role of Aegle marmelos on aspirin-induced gastro-duodenal ulceration in albino rat model: A possible involvement of antioxidants. Saudi Journal of Gastroenterology, 18(3), 188. https://doi.org/10.4103/1319-3767.96452

[8] Seo, P. J., Kim, N., Kim, J. H., Lee, B. H., Nam, R. H., Lee, H. S., Park, J. H., Lee, M. K., Chang, H., Jung, H. C., & Song, I. S. (2012, April 15). Comparison of Indomethacin, Diclofenac and Aspirin-Induced

Gastric Damage according to Age in Rats. Gut And Liver, 6(2), 210–217. https://doi.org/10.5009/gnl.2012.6.2.210

[9] Hegde, H., Kholkute, S., Patil, P., Roy, S., & Kumar, D. (2013). Antiulcer activity of water-soaked Glycine max L. grains in aspirin induced model of gastric ulcer in Wistar rats. Journal of Ayurveda and Integrative Medicine, 4(3), 134. https://doi.org/10.4103/0975-9476.118679

[10] Nawale, S., Priyanka, N., Das, S., & Ganga Raju, M. (2019, April). Data of in vivo screening of antiulcer activity for methanolic extract of Vernonia elaeagnifolia DC. Data in Brief, 23, 103753. https://doi.org/10.1016/j.dib.2019.103753

[11] Reddy, V. T., Kumar, S. H. H., & Bakshi, V. (2016, March 1). Antiulcer Effect of Formulation of Aloe Vera and Liquorice Againt Aspirin Induced Peptic Ulcer. INTERNATIONAL JOURNAL OF APPLIED PHARMACEUTICAL SCIENCES AND RESEARCH, 1(01), 42–45. https://doi.org/10.21477/ijapsr.v1i1.10174

[12] Bhandari U., Khushtar, M., Kumar, V., & Javed, K. (2009). Protective effect of ginger oil on aspirin and pylorus ligation-induced gastric ulcer model in rats. Indian Journal of Pharmaceutical Sciences, 71(5), 554. https://doi.org/10.4103/0250-474x.58195

[13] Bandonienė, D., Murkovic, M., Pfannhauser, W., Venskutonis, P., & Gruzdienė, D. (2002, February). Detection and activity evaluation of radical scavenging compounds by using DPPH free radical and online HPLC-DPPH methods. European Food Research and Technology, 214(2), 143–147. https://doi.org/10.1007/s00217-001-0430-9

[14] Mishra, K., Ojha, H., & Chaudhury, N. K. (2012, February). Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. Food Chemistry, 130(4), 1036–1043. https://doi.org/10.1016/j.foodchem.2011.07.127

[15] Ali, B. M., Boothapandi, M., & Sultan Nasar, A. (2020, February). Nitric oxide, DPPH and hydrogen peroxide radical scavenging activity of TEMPO terminated polyurethane dendrimers: Data supporting antioxidant activity of radical dendrimers. Data in Brief, 28, 104972. https://doi.org/10.1016/j.dib.2019.104972

[16] Chaudhary, P., & Janmeda, P. (2022, November 15). Comparative pharmacognostical standardization of different parts of Euphorbia neriifolia Linn. Vegetos, 36(1), 210–221. https://doi.org/10.1007/s42535-022-00508-x

[17] Krishnaswamy, T., Subramaniam, P., Sellamuthu, M., & Krishnamoorthy, K. (2014). Quantification of phytochemicals and in vitro antioxidant potential of various solvent extracts of certain species of Acanthaceae. International Journal of Green Pharmacy, 8(1), 58. https://doi.org/10.4103/0973-8258.126826

[18] Dasari, N. P., Ganga Rao, B., Sambasiva Rao, E., Mallikarjuna Rao, T., & Praneeth D, V. (2012, April). Quantification of phytochemical constituent's in vitro antioxidant activity of Synadium grantii. Free Radicals and Antioxidants, 2(2), 68–72. https://doi.org/10.5530/ax.2012.2.2.12

[19] Chellappan, D. R., Joseph, J., & Balasubramanian, P. (2011, December). Pharmacognostical, antioxidant and antiulcer screening of Cyclea peltata roots. Revista Brasileira De Farmacognosia, 21(6), 1096–1103. https://doi.org/10.1590/s0102-695x2011005000168

[20] Al Batran, R., Al-Bayaty, F., Jamil Al-Obaidi, M. M., Abdualkader, A. M., Hadi, H. A., Ali, H. M., & Abdulla, M. A. (2013, May 28). In Vivo Antioxidant and Antiulcer Activity of Parkia speciosa Ethanolic Leaf Extract against Ethanol-Induced Gastric Ulcer in Rats. *PLoS ONE*, *8*(5), e64751. https://doi.org/10.1371/journal.pone.0064751